

ANTIOXIDANT AND ANTI-HEMOLYTIC ACTIVITIES OF PHENOLIC CONSTITUENTS OF SIX MOROCCAN DATE FRUIT (*PHOENIX DACTYLIFERA L.*) SYRUPS

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Date fruits are traditionally used to prepare a wide range of products such as vinegar wine, jam and syrup named locally “*Tahlawt*” moreover, their direct consumption. The aim of this study is to investigate the antioxidant and anti-hemolytic activities of six syrups prepared traditionally from six different date fruit cultivars grown in southeast Morocco. Significant difference ($P < 0.05$) was established among analysed syrups. The highest phenolic (6.70 g GAE /100g DW) and flavonoid content (932.82 mg RE /100g DW) were found in *Jihl* syrups which possessed the highest antioxidant activity based on FRAP (9.55 mmol TE/100g DW), ABTS (8.27 mmol TE /100 g DW), DPPH_{IC50} (381.99 µg/mL) and exhibited the highest membrane protective effect (317.70 min). *Tamaajount* syrup contains the lowest phenolic (3.72g GAE /100g DW) and flavonoid content (528.19 mg RE /100g DW) and presented the lowest antioxidant activity based on FRAP (5.30 mmol TE/100g DW), ABTS (4.68 mmol TE /100 g DW) and DPPH_{IC50} (1.095 mg/mL) as well as the lowest membrane protective effect (226.44 min). The results obtained suggest that date fruit syrups could be considered as a functional food or functional food ingredient because of their high phenolic compounds, which act as antioxidants and membrane stabilizer.

Keywords: syrups, antioxidant, antihemolytic, phenolic content

INTRODUCTION

Morocco occupies the 12th in the world and 9th Arab-largest producer of dates with an annual production above 113,000 tons (FAOSTAT 2012). Around, 40% of this production is constituted from low-quality cultivars (Sedra, 2003). These important quantities of low quality dates are sold at low prices or integrated in animal feed because of too hard texture. Although they still considered as a good source of sugars, minerals and other substances (Ramadan, 1995). The oasis populations have developed a methods for processing this low-quality dates in various products more profitable and more appreciated by consumers such as syrup locally known as “*Tahlawt*”.

The aim of this research was to evaluate the antihemolytic activity, antioxidant potential and phenolics content of six Moroccan date fruit syrups. Expectantly, this research work will provide efficient practical evidence of antioxidant potential for future development and utilization of these syrups.

MATERIALS AND METHODS

Preparation of date fruit syrups: Six Moroccan date fruits varieties (Bouslikhen, Bousthammi, Iklan, Jihl, Lhafs and Tamaajount,) at low quality (hard texture or poorly conserved because of high content on water). The date fruits

were pitted, crushed and cut to small pieces with a sharp knife. The result date fruit pieces was mixed with water at 1:3 ratio and left overnight to facilitate the extraction. The result mixture was boiled for about 2 hours than filtered through a cloth and water is again added to the presscake and the mixture is boiled again. This extraction is repeated 3 times, than the collected juice is concentrated by boiling to increase the Brix.

Preparation of rich polyphenol extracts: The rich Phenolic compounds extract was prepared according to the method of (Bouhlali *et al.*, 2015) with slight modifications. Briefly, 30 g of date fruit syrup was extracted with 150 ml acetone–water (4:1, v/v), at 35°C for 12 h using an orbital shaker-incubator. The mixture was then filtered and the filtrate was concentrated under reduced pressure at 40°C until the total evaporation of solvent, using a rotary evaporator. The results acetonc crude extract were kept at -20°C in dark glass bottles until use. The extract was dissolved in the water in known dilution to determine phenolic, flavonoids and condensed tannins content and their antioxidant capacity was evaluated using the same dilution.

Measurement of total phenolic compounds: The total phenolic contents in date fruit syrups were determined according to the method described by the International Organization for Standardization (ISO 14502-1).

Measurement of flavonoid content: The total flavonoid content of date fruit syrups was determined by the method of (Kim *et al.*, 2003).

Measurement of total condensed tannins: Total condensed tannins were determined using method described by Heimler *et al.* (2006).

ABTS radical scavenging assay: The ABTS radical scavenging was measured using the method of (Re *et al.*, 1999).

Ferric reducing antioxidant power assay: The ferric reducing activity of date fruits syrup extract was estimated based on the method of (Benzie and Strain, 1999).

DPPH radical scavenging activity: Scavenging radical activity of date fruit syrups against stable DPPH was assessed as described by (Blois 1958) method with slight modifications.

The protective effect date fruit syrups against AAPH induced erythrocyte oxidative hemolysis

The anti-hemolytic activity induced by a peroxy radical initiator, AAPH was measured according to the method established by Blache and Prost (1992) with minor modifications. Two hundred microliters of Rabbit blood collected in heparin bulbs was mixed with 10 μ L of date fruit extract and then 600 μ L of AAPH (10%) was added. The mixture was incubated at 37°C. The absorbance of the mixture was measured at 450 nm every 5 min. The date fruit syrup extract was replaced by Trolox and saline (0.9% NaCl) in the positive and negative control, respectively. The Protective effects of date fruit extract on free radical induced hemolysis of erythrocytes were estimated from the time required for half-hemolysis.

Statistical analysis: Statistical analysis was performed using StatView 5.0 software. The experimental results were reported as mean \pm SE (standard error) (n=5) on a dry weight. Analysis of variance (ANOVA) and post-hoc Bonferroni. ($p < 0.0018$) tests were used to compare the experimental groups. Pearson's correlation coefficient (r) was used to measure the association between two variables. Differences at $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Fruits and vegetable Phenolic contents: Phenolic compounds are abundant parts of plants and their major sources in human food are various beverages, fruits and vegetables. Acetone is commonly used solvents and was found to be more efficient for phenolic content extracting from date fruit (Kchaou *et al.*, 2013). In order to evaluate the antioxidant potential of the extracts from date fruit syrups, it was realistic approach to find the constituents of several polyphenols in liquid acetone.

Table 1 illustrate the phenolic, flavonoid, and condensed tannins content of analyzed date fruit syrups. Among these

date fruit syrups, the highest phenolic content (6.70 g GAE/100 g of syrup dry weight) was observed from *Jihl* syrup, however, *Tamaajount* syrup exhibited the lowest phenolic contents (3.72 mg GAE/100 g syrup dry weight). On the other hand Total flavonoid contents (TFC) ranged over 528.19 – 932.82 mg RE/100 g of syrup DW. Highest TFC was recorded for *Jihl* syrups while the lowest was for *Tamaajount* syrup. Our results are very higher compared with previous investigations who found that phenolic content and flavonoid content of date fruit syrup ranged between 368.35 - 529.28 GAE/100 g FW and 39.56 - 194.51 mg CE/100g of syrup FW, respectively for (Abbès *et al.*, 2013) and between 434.3 -769.6 CE mg/100 g FW and between 310.5 - 554.0 mg QE/ 100 g for, respectively (Al-Mamary *et al.*, 2010). This differences in phenolic content may be due to the method of syrup preparation include temperature, time of incubation, pH as well as variety. Regarding condensed tannins content the highest amount of these compound was showed in *Lhafs* syrup whereas *Iklane* syrup had the lowest values. The important amount of phenolic content depicted in the syrups, compared to date fruit which contain an amount of phenolic and flavonoid ranged respectively between 331.86-537.07 mg GAE/100g DW and 68.88 - 208.53 mg of RE/100 g DW. Bouhlali *et al.*, 2015 show that the thermal processes during syrups preparation may lead to improve the extraction of phenolic compounds from date fruit. The same observation on the effect of temperature of phenolic content was already observed (Chandrasekara & Shahidi, 2011; Yu *et al.*, 2005). However, the low amount of flavonoid observed in this study compared to total phenolic content could be due to their degradation during the boiling at syrups preparation the same observation was reported by Viña & Chaves (2008). They observed a loss of about 22% in total flavonoids in boiled celery at a temperature of 50°C during 90s.

Evaluation antioxidant activities: Phenolics like phenolic acids, tannins and flavonoids are well-thought-out to be major contributors to the antioxidant potential (Velioglu *et al.*, 1998). The determination of the antioxidant potential of date fruit syrups required different methods because of their chemical complexity. In the present study, therefore, three complementary methods were followed to evaluate the reducing ability and the capacity to scavenge free radicals.

The free radical-scavenging activity of analyzed date fruit syrups is shown in **Table 2**. The highest scavenging activity based on ABTS assay (8.27 mmol TE/100g of syrups DW) and DPPH assay (381.99 μ g of syrup DW/mL) was found in *Jihl* syrup. The lowest scavenging ability based on ABTS assay (4.68 mmol TE/100g of syrups DW) and DPPH assay (1095.58 μ g of syrup DW/mL) was depicted in *Tamaajount* syrup.

The FRAP assay is a simple, inexpensive and widely employed method used to evaluate of antioxidant capacity of medicinal plants (Li *et al.*, 2008) which, is based on the ability of antioxidants to reduce ferric (Fe³⁺) ions to ferrous (Fe²⁺) ions in the presence of TPTZ, forming an intense

Table 1. Total phenolic, flavonoids and condensed tannins content of studied date fruit syrup.

	TPC g GA/ 100g DW	TFC mg RE/ 100gDW	CTC g CE/ 100gDW
<i>Bousthammi</i> syrup	5.84 ± 0.31 ^a	758.68 ± 11.27	3.82 ± 0.14
<i>Bouslikhen</i> syrup	5.39 ± 0.17	664.31 ± 10.42 ^a	3.19 ± 0.23 ^{bc}
<i>Iklane</i> syrup	4.52 ± 0.34	655.71 ± 8.62 ^a	3.32 ± 0.11 ^c
<i>Jihl</i> syrup	6.70 ± 0.25	932.82 ± 9.62	2.48 ± 0.09 ^a
<i>Lhafs</i> syrup	5.96 ± 0.24 ^a	863.57 ± 9.38	2.26 ± 0.13 ^a
<i>Tamaajount</i> syrup	3.72 ± 0.12	528.19 ± 6.81	2.87 ± 0.19 ^b

Values in average (n=5) ± SE .Averages, in the same column, with same letters are not significantly different using post hoc Bonferroni tests (p < 0.0018). TPC: Total Phenolic content; TFC: Total Flavonoid content; CTC: Condensed Tannins content

Table 2. Antioxidant activities of analysed date fruit syrups

	FRAP mmol TE /100g DW	ABTS mmol TE /100gDW	DPPH µg/mL
<i>Bousthammi</i> syrup	7.08 ± 0.33	6.23 ± 0.21 ^b	760.59 ± 22.20
<i>Bouslikhen</i> syrup	7.69 ± 0.20	6.57 ± 0.19 ^b	562.83 ± 34.21
<i>Iklane</i> syrup	5.98 ± 0.24	5.46 ± 0.17	649.34 ± 45.64
<i>Jihl</i> syrup	9.55 ± 0.13	8.27 ± 0.34 ^a	381.99 ± 17.98
<i>Lhafs</i> syrup	9.09 ± 0.27	7.93 ± 0.27 ^a	447.31 ± 6.28
<i>Tamaajount</i> syrup	5.30 ± 0.17	4.68 ± 0.22	1095.58 ± 56.37

Values in average (n=5) ± SE .Averages, in the same column, with same letters are not significantly different using post hoc Bonferroni tests (p < 0.0018)

Table 3. Correlation phenolic and flavonoid content with antioxidant activities

	TPC	TFC	CT	FRAP	ABTS	DPPH	AhE
TPC	1						
TFC	0.899	1					
CT	0.067	0.200	1				
FRAP	0.864	0.869	0.328	1			
ABTS	0.869	0.906	0.328	0.994	1		
DPPH	0.703	0.722	0.171	0.786	0.811	1	
AhE	0.931	0.926	0.220	0.936	0.939	0.720	1

TPC: total phenolic content; TFC: Total flavonoids content CT: condensed tannins AhE: anti-hemolytic effect

blue ferrous (Fe²⁺) -TPTZ complex at an acid pH (3.6). The change is monitored spectrophotometrically at 593nm (Huang *et al.*, 2005). As seen from the data in the table, the FRAP values varied from 5.30 to 9.55 mmol TE/100g of syrups DW. In general, the studied date fruit syrups had very high antioxidant capacities. The highest FRAP value was observed in *Jihl* syrup and *Tamaajount* syrup had the lowest value.

Correlation between antioxidant capacities, phenolic content, flavonoids and condensed tannins content: Correlation analysis was used to explore the relationships amongst antioxidant capacities, total phenolic, flavonoids and condensed tannins measured for all the syrup samples (Table 3). The result showed a positive linear correlation between the antioxidant capacities and total phenolic content ranged between $R^2 = 0.703$ for DPPH assay and $R^2 = 0.869$ for ABTS assay which is better than the correlation between flavonoids and antioxidant activity ranged between $R^2 = 0.722$ for DPPH assay and $R^2 = 0.906$ for ABTS assay. However, very low correlation was found between condensed

Table 4. Protective effect of date fruit syrup against AAPH induced erythrocyte hemolysis

	Hemolysis half-time (min)
Control	105.66 ± 3.46 ^a
AAPH + blood	52.17 ± 2.61
AAPH +blood+ <i>Bouslikhen</i> syrup	267.13 ± 7.83 ^b
AAPH +blood+ <i>Jihl</i> syrup	317.79 ± 10.63
AAPH +blood+ <i>Bousthammi</i> syrup	269.49 ± 9.02 ^b
AAPH +blood+ <i>Tamaajount</i> syrup	226.44 ± 7.59
AAPH +blood+ <i>Lhafs</i> syrup	291.86 ± 9.76
AAPH +blood+ <i>Iklane</i> syrup	244.37 ± 8.21
AAPH+ Trolox 1%	109.73 ± 5.29 ^a

Values in average (n=5) ± SE .Averages, the column with the same letters are not significantly different using post hoc Bonferroni tests (p < 0.0018)

Table 5. Evaluation of hemolysis induced by date fruit syrup extracts

	Hemolysis half-time (min)
Control	105.66 ± 3.46
Sang + Trolox	118.25 ± 6.29
Blood + <i>Bouslikhen</i> syrup	271.64 ± 5.72
Blood+ <i>Jihl</i> syrup	294.17 ± 5.21 ^a
Blood+ <i>Bousthammi</i> syrup	257.03 ± 7.24 ^b
Blood+ <i>Tamaajount</i> syrup	238.03 ± 6.82 ^c
Blood+ <i>Lhafs</i> syrup	293.20 ± 5.92 ^a
Blood+ <i>Iklane</i> syrup	247.52 ± 7.62 ^{bc}

Values in average (n=5) ± SE .Averages, the column with the same letters are not significantly different using post hoc Bonferroni tests (p < 0.0018).

tannins content and antioxidant activity varied between $R^2 = 0.171$ for DPPH assay and $R^2 = 0.328$ for FRAP assay, therefore, flavonoids and phenolic acid compounds are the dominant contributor to the antioxidant activity. The strong correlation in this study confirm by several studies (Chang *et al.*, 2001).

Concerning the relationships between antioxidant assays the positive linear correlation between them which varied between $R^2 = 0.786$ for DPPH/FRAP and $R^2 = 0.994$ for FRAP/ABTS, suggested that antioxidant components in these date fruit syrups could reduce oxidants (such as ferric ions) and scavenge free radicals. The strongest correlation between FRAP and ABTS assays may be due to the same mechanism that they have and their similar redox potential 0.70 V for Fe(II)/(III) and 0.68 V for ABTS/ABTS+• (Müller *et al.*, 2011). The difference of redox potential between ABTS and FRAP assay justified the high antioxidant activities depicted using FRAP assay than ABTS assay that means that any compound with lower Fe (II)/(III) redox potential can theoretically reduce Fe (III) to Fe (II) and contributes to the FRAP values resulting in falsely high FRAP values.

The Anti-hemolytic effect of date fruit syrups: Erythrocytes membrane lipids are rich in polyunsaturated fatty acids and therefore the exposure to free radical generated from the degradation of AAPH at physical temperature is the reason for hemolysis (Zou *et al.*, 2001).

Table 4 shows the protective effects of date fruit syrups and Trolox on the hemolysis induced by AAPH. This effect was

found to be dose dependent in all date fruit syrups. The highest protective effect was found in *Jihlsyrups*, which possessed the highest half hemolysis value (317.79 min) whereas, the lowest half hemolysis value (226.44 min) was found using *Tamaajountsyrups*. The important half hemolysis value (52.17min) illustrated in the negative control may be due to the endogenous antioxidants in the erythrocytes which can trap radicals to protect them against free radical induced hemolysis as described previously (Zou *et al.*, 2001).

The high half hemolysis value observed for all date fruit syrups compared to negative control show that these extracts did not provide just the protective effect but also the stabilizing effect of erythrocytes membrane. The non-significant hemolysis observed when erythrocytes were treated only with date fruit syrups as illustrated in **Table 5** can be justified as nontoxic and harmless for the cells. The high positive correlation between phenolic/erythrocyte protective effect ($R^2 = 0.931$), flavonoids/erythrocyte protective effect ($R^2 = 0.926$) show that phenolic and flavonoid content are the main contributor to the erythrocytes protective effect through their AAPH scavenging activity as show the high correlation between both DPPH and ABTS in one hand and protective effect. Our results are in agreement with other studies showing that polyphenols are able to protect erythrocytes from oxidative stress or increase their resistance to damage caused by oxidants (Carvalho *et al.*, 2010 and Mendes *et al.*, 2011).

CONCLUSION

The results presented showed the six date fruit syrups are very rich on phenolic, flavonoid and condensed tannins content and exhibited the high antioxidant activity and very important protective effect of membrane erythrocytes against AAPH induced hemolysis. It is due to the high antioxidant activity of the date fruit syrups is considered a very good functional food ingredient as well as an appropriate source in pharmaceutical field.

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